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Enterovirus infections in England and Wales, 2000–2011: the impact of increased molecular diagnostics

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Abstract

There have recently been significant changes in diagnostic practices for detecting enterovirus (EV) infections across England and Wales. Reports of laboratory-confirmed EV infections submitted by National Health Service (NHS) hospital laboratories to Public Health England (PHE) over a 12-year period (2000–2011) were analysed. Additionally, the PHE Virus Reference Department (VRD) electronic database containing molecular typing data from 2004 onwards was interrogated. Of the 13 901 reports, there was a decline from a peak of 2254 in 2001 to 589 in 2006, and then an increase year-on-year to 1634 in 2011. This increase coincided with increasing PCR-based laboratory diagnosis, which accounted for 36% of reported cases in 2000 and 92% in 2011. The estimated annual incidence in 2011 was 3.9/100 000 overall and 238/100 000 in those aged <3 months, who accounted for almost one-quarter of reported cases (n = 2993, 23%). During 2004–2011, 2770 strains were submitted for molecular typing to the VRD, who found no evidence for a predominance of any particular strain. Thus, the recent increase in reported cases closely reflects the increase in PCR testing by NHS hospitals, but is associated with a lower proportion of samples being submitted for molecular typing. The high EV rate in young infants merits further investigation to inform evidence-based management guidance.

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Introduction

The widespread introduction of highly effective conjugate vaccines in industrialized countries has led to a significant reduction in the burden of serious bacterial infections across all age groups through direct and indirect (herd) protection [1,2]. As a consequence, most individuals with central nervous system infections now have aseptic meningitis [3,4], which is usually of viral aetiology [5,6]. In children, aseptic meningitis now accounts for >90% of meningitis cases [3,7], and *c.* 85% of these are caused by enteroviruses (EVs) [5].

Almost 100 EV types have been identified, accounting for 30 000–50 000 annual hospital admissions in the USA, mainly for aseptic meningitis [8]. The vast majority of EV infections occur in children aged <5 years, with one-third of cases being diagnosed in infants aged <12 months [9,10]. The clinical manifestations of EV infection vary from non-specific febrile illnesses and respiratory symptoms to serious presentations including meningitis, encephalitis, myocarditis, hepatitis, and potentially fatal multi-organ failure [11].

With the increasing availability of PCR testing, many hospital laboratories no longer perform viral cultures routinely. PCR testing is cheaper, rapid, requires small amounts of biological material, can be used for multiple pathogens, and can detect almost all EV types, because the viruses share common genetic sequences. For EVs, PCR has greater sensitivity and specificity than viral cultures [12–15]. Data from these studies show that it is possible to deliver a result within 5–6 h of sample testing, which could, in turn, deliver significant cost

savings by reducing unnecessary antimicrobial use and shortening hospital stays.

PCR testing, however, requires clinicians to specifically request PCR testing for EVs, and strains need to be submitted to reference centres for molecular typing. At a national level, EV surveillance is important to identify emerging strains, link EV types with clinical patterns of disease, and help develop evidence-based guidance to manage EV infections, including the need for new therapies. This study aimed to describe the epidemiology of laboratory-confirmed EV infections in England and Wales over a 12-year period, with particular emphasis on the impact of changing diagnostic practices within National Health Service (NHS) hospital laboratories in recent years.

Methods

NHS hospitals in England and Wales provide free and unrestricted medical care for the entire population of *c.* 55 million individuals, and their microbiology laboratories routinely report clinically significant infections electronically on a voluntary basis to Public Health England (PHE) using LabBase2, which is subject to weekly audits of participation and data quality. For this study, demographic (age, gender, and geographical region) and strain-specific (sample type, diagnostic method, date of detection, and serotype) records of all positive EV strains from different biological sites (blood, cerebrospinal fluid (CSF), gastrointestinal or respiratory) during 2000–2011 were extracted from LabBase2, de-duplicated, and analysed. Reporting of the same pathogen from a patient within 14 days was considered to be a single episode.

As part of the PHE's active polio surveillance commitment to the WHO, NHS laboratories are requested to submit all positive EV strains to the PHE Virus Reference Department (VRD) for confirmation and molecular typing, as described previously [16]. This is offered as a free national service. Typing results are reported back to the laboratories, but are often not entered into the local database, and so molecular data are rarely captured in LabBase2. We therefore interrogated the VRD electronic database, which contains molecular typing data for all submitted strains from 2004 onwards. LabBase2 and the VRD database are two exclusive datasets.

Data analysis

Data were analysed with STATA, v.11 (StataCorp, College Station, TX, USA). Cases were categorized into the following age groups: 0–3 months, 3–11 months, 1–4 years, 5–14 years, 15–24 years, 25–44 years, 45–64 years, and ≥65 years. Categorical variables were expressed as proportions, and compared by use of the chi-square test or Fisher exact test, as appropriate. Age-specific incidence was calculated from mid-year population estimates obtained from the Office for National Statistics (www.statistics.gov.uk).

Results

During 2000–2011, a total of 13 901 laboratory-confirmed EV cases were reported in England and Wales (mean annual incidence, 2.9/100 000). The number of reports increased from 2000 to 2001, and then declined to its lowest level in 2006, before increasing year-on-year until 2011 (Fig. 1). These changes occurred proportionally across all age groups, with no particular age group dominating during the 12-year surveillance period (Fig. 1). The characteristic seasonal trends in EV—peaks in the summer and troughs in the winter—were observed only during periods of high incidence in 2000 and 2010 (Fig. 2).

Over the 12-year period, the proportion of samples confirmed by PCR increased rapidly from 36% in 2000 to 92% in 2011 (45% overall), whereas diagnosis by culture (53% in 2000 to 31% in 2006 and 3% in 2011), microscopy (3% in 2000 to 0% in 2011) and serology (8% in 2000 to 2% in 2011) declined proportionally across the age groups (Fig. 3). For the

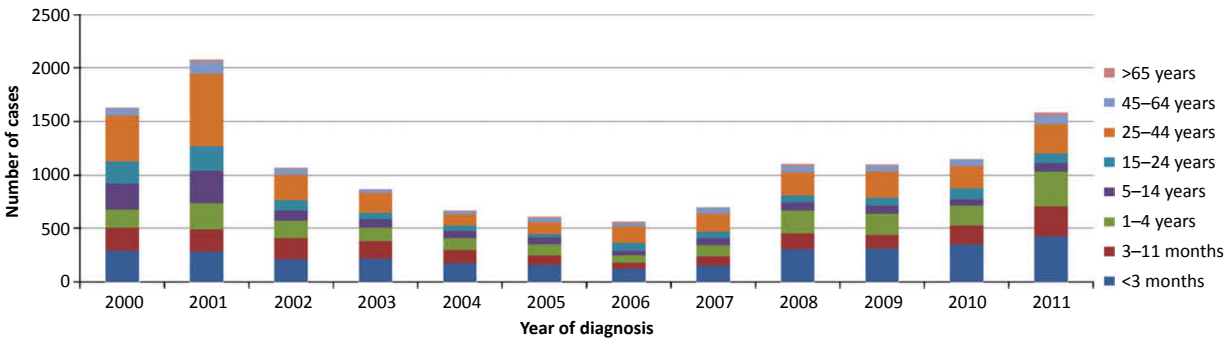

FIG. 1. Age distribution of laboratory-confirmed enterovirus infections in England and Wales during 2000–2011 obtained from LabBase 2.

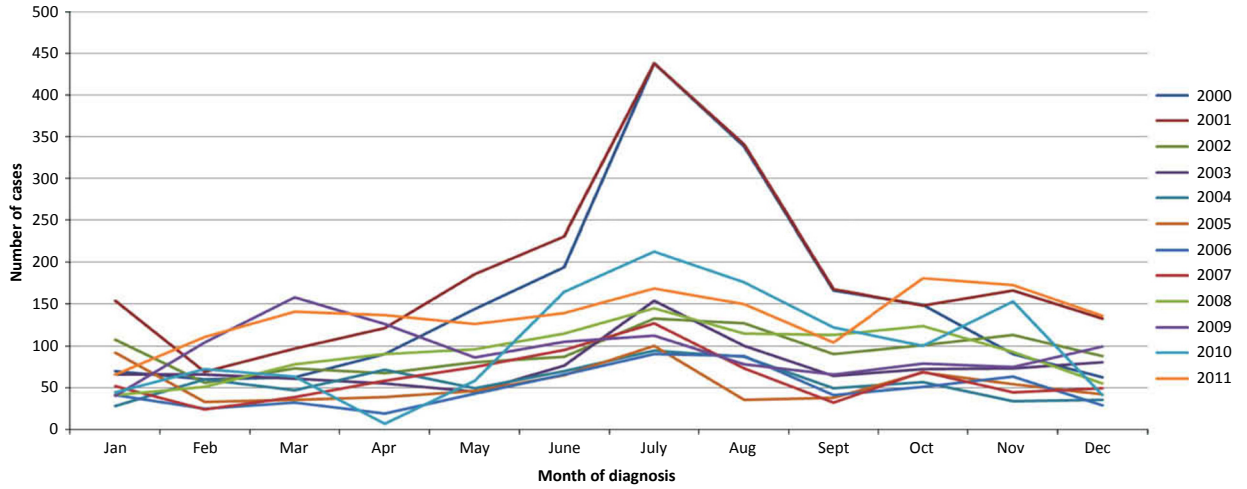

FIG. 2. Enterovirus seasonal trends in England and Wales during 2000–2011 obtained from LabBase 2.

13 114 (94%) cases with age reported, the median age at diagnosis was 4.0 years (interquartile range 0.3–28.5 years). More than half (n = 7606, 58%) were diagnosed in children (<15 years). The incidence was significantly higher in children than in adults (7.0/100 000 vs. 1.4/100 000; p <0.0005). In particular, young infants (<3 months) accounted for 23% of all EV infections and 37% of childhood EV infections, and had the highest incidence of EV infection (Table 1). After this age, the incidence declined rapidly, with a smaller peak among those aged 25–44 years (Table 1).

In contrast to changes in the methods used for EV detection, the sources of the samples tested and subsequently reported through LabBase 2 remained constant, with CSF accounting for 36% of samples (n = 5032), respiratory specimens for 26% (n = 3563), gastrointestinal specimens for 17% (n = 2394), and blood specimens for 12% (n = 1602). The proportion of CSF-positive cases, however, was higher in some age groups: 25–44 years (64%), 15–24 years (57%), and <3 months (46%).

Molecular typing results were recorded in LabBase2 for only 28% (3921/13901) of EV strains, and the proportion of reports with typing results declined with age and over time. Of the typed strains, 55% (n = 2143) were echoviruses, 23% (n = 904) Coxsackie B, 5% (n = 194) Coxsackie A, 0.9% (n = 351) EV type 70, and 0.6% (n = 24) EV type 71 (Fig. 4). Nearly all polioviruses were detected in infants aged <3 months (283/560, 51%) or toddlers (232/560, 41%), and all were confirmed by the VRD to be the oral poliovirus vaccine (OPV) strain. The last vaccine-related poliovirus infection was detected in 2007.

The 2000–2001 peak was attributable to an echovirus 13 outbreak, accounting for 62% (459/742) and 73% (774/1066) of strains typed in 2000 and 2001, respectively. The increasing PCR diagnosis of clinical specimens coincided with a lower proportion of molecular typing data for EV in LabBase2. The proportion of strains without typing information in LabBase2 increased from 72% (424/588) in 2006 to 94% (1540/1633) in 2011. More complete genotyping data were available from the VRD database. Table 2 summarizes the source of clinical specimen by EV type. During 2004–2011, 2770 strains underwent molecular typing, although typing failed for 32% (895/2770 strains), usually because of small sample volumes or low viral loads (Table 3). The available typing data suggest natural fluctuations in circulating EV strains, with no evidence of predominance of any particular EV strain over the course of the surveillance period. Echoviruses were the predominant group for several years (2004, 2009, and 2011).

Discussion

This study is the first to report the impact of changing diagnostic practices for EV detection in England and Wales since 1994 [9]. Currently, very few NHS laboratories perform light/electron microscopy or viral cell culture, and most EV cases are diagnosed by PCR. The increase in EV reporting in recent years closely reflects the increase in PCR testing, which was also associated with a lower proportion of samples being submitted to the national reference department for confirmation and typing. The high sensitivity of PCR as compared with cell culture will also have contributed to the number of laboratory-confirmed cases [12–15], and this trend is likely to continue as more sensitive multiplex assays for simultaneous detection of multiple pathogens in biological samples are adopted by local hospitals. In addition to being cheap, rapid, and sensitive, PCR testing for EV can directly impact on individual patient care by reducing unnecessary antibiotic usage

TABLE 1. Estimated incidence of enterovirus infection by age group and year in England and Wales during 2000–2011 obtained from LabBase2, an electronic database used by National Health Service hospital laboratories to voluntarily report clinically significant infections to Public Health England (numbers of cases in parentheses)

	Enterovirus infection incidence during 2000–2011											
Age group	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**Total**
<3 months	193.2 (293)	190.8 (281)	144.1 (212)	143.7 (218)	108.1 (170)	97.0 (155)	75.3 (123)	89.5 (151)	174.1 (306)	177.4 (310)	197.6 (351)	238.1 (423)	153.3 (2993)
3–11 months	46.6 (212)	47.3 (209)	45.1 (199)	35.2 (160)	27.8 (131)	19.0 (91)	11.8 (58)	18.0 (91)	28.1 (148)	24.8 (130)	32.6 (174)	52.4 (279)	32.1 (1882)
1–4 years	6.8 (173)	9.8 (244)	6.5 (158)	5.3 (127)	4.5 (107)	4.3 (103)	2.6 (63)	4.1 (103)	8.3 (216)	7.5 (199)	6.8 (185)	11.9 (325)	6.6 (2003)
5–14 years	3.6 (240)	4.6 (307)	1.5 (101)	1.3 (84)	1.0 (65)	1.0 (67)	0.8 (51)	1.0 (62)	1.2 (75)	1.2 (76)	1.0 (60)	1.3 (82)	1.6 (1270)
15–24 years	4.8 (208)	5.2 (229)	2.2 (96)	1.3 (56)	1.2 (54)	0.7 (29)	1.6 (73)	1.4 (62)	1.5 (67)	1.5 (72)	2.2 (103)	1.9 (90)	2.1 (1139)
25–44 years	10.9 (426)	17.1 (679)	5.9 (238)	4.6 (185)	2.6 (104)	2.8 (115)	3.6 (151)	4.0 (171)	5.0 (213)	5.7 (246)	4.8 (211)	6.2 (275)	6.0 (3014)
45–64 years	0.6 (70)	1.0 (107)	0.5 (55)	0.3 (31)	0.2 (28)	0.3 (37)	0.3 (34)	0.5 (48)	0.5 (59)	0.4 (51)	0.4 (59)	0.6 (82)	0.5 (661)
≥65 years	0.1 (10)	0.2 (21)	0.1 (7)	0.1 (8)	0.1 (8)	0.1 (14)	0.1 (11)	0.1 (7)	0.2 (17)	0.1 (10)	0.1 (9)	0.3 (30)	0.1 (152)
Not recorded	231	177	56	44	14	36	25	43	16	34	63	48	787
Total [a]	4.8 (1863)	5.8 (2254)	2.9 (1122)	2.3 (913)	1.7 (681)	1.6 (647)	1.5 (589)	1.9 (738)	2.7 (1117)	2.7 (1128)	2.9 (1215)	3.9 (1634)	2.9 (13 901)
<1 year	83.2 (505)	83.2 (490)	69.8 (411)	62.3 (378)	47.9 (301)	38.5 (246)	27.7 (181)	35.9 (242)	64.6 (454)	62.9 (440)	73.9 (525)	98.8 (702)	62.4 (4875)
<5 years	21.5 (678)	23.7 (734)	18.8 (569)	16.8 (505)	13.5 (408)	11.4 (349)	7.8 (244)	10.8 (345)	20.3 (670)	19.0 (639)	20.6 (710)	29.8 (1027)	18.0 (6878)
<15 years	9.3 (918)	10.6 (1041)	6.9 (670)	6.1 (589)	4.9 (473)	4.3 (416)	3.1 (295)	4.3 (407)	7.8 (745)	7.5 (715)	8.0 (770)	11.5 (1109)	7.0 (8148)
≥15 years	2.5 (714)	3.6 (1036)	1.3 (396)	0.9 (280)	0.6 (194)	0.6 (195)	0.9 (269)	1.0 (288)	1.1 (356)	1.2 (379)	1.2 (382)	1.5 (477)	1.4 (4966)

[a]Total number of cases includes those with age not recorded.

and shortening hospital stays. A study in Marseille, for example, demonstrated that, following the introduction of routine PCR testing, durations of hospitalization were reduced from 5.4 days to 2.2 days during two separate outbreaks of EV type 30, resulting in an estimated cost-saving of €322 000 [17].

Interrogation of two national datasets contemporaneously allowed us to determine the proportion of samples being submitted for molecular typing nationally. Whereas the increase in PCR testing within NHS hospital microbiology laboratories has improved EV diagnosis in recent years, the proportion of strains with molecular typing data in LabBase2 has declined significantly. Typing information held by the VRD, however, showed that the absolute number of strains undergoing molecular typing increased from 113 in 2004 to 698 in 2011. This increase was, in part, attributable to active efforts by the VRD to increase submission rates from NHS laboratories as part of polio surveillance.

In 2000–2001, the UK, other European countries, Australia and the USA experienced an outbreak of echovirus infection, with echovirus 13 dominating in 2000 and echovirus 30 in 2001 [18,19]. Echovirus 30 is one of the more common echovirus genotypes; it causes outbreaks every few years, and causes mainly meningoencephalitis in children and adults, with varying clinical severity [20]. Echovirus 13 is a rare genotype that was responsible for major global outbreaks in the early 2000s [18,21]. The UK was declared free of wild poliovirus in 1998, and Europe followed suit in 2002. In 2004, the UK switched from live OPV to inactivated poliovirus vaccine in the infant immunization schedule. In England and Wales, the VRD plays a key role in investigating all polioviruses identified by NHS laboratories as part of ongoing polio elimination surveillance, to ensure that that these are not wild-type polioviruses or neurovirulent, vaccine-derived strains. In recent years, the detected strains have usually been from children from overseas who had recently received an OPV. In the past 12 months, however, there have been reports of polio in nine countries, with 68 cases being recorded in the first months of 2014 as compared with 24 cases in the same period of 2013 [22]. In May this year, the WHO declared the spread of polio to be an international public health emergency, thus highlighting the need for ongoing active national polio surveillance.

In the UK and the USA, surveillance data from the 1980s to 2005 have shown annual fluctuations in circulating EV types, with a seasonal variation [8–10]. Coxsackie A9 and B2, along with echoviruses 3, 7, 13, and 16, have all shown annual peaks and troughs in different years [8,9]. Neither molecular typing results in LabBase2 or the VRD data indicate a predominance of any particular EV type in recent years, suggesting that the observed increase in EV reports most likely reflects increased PCR testing by NHS hospital

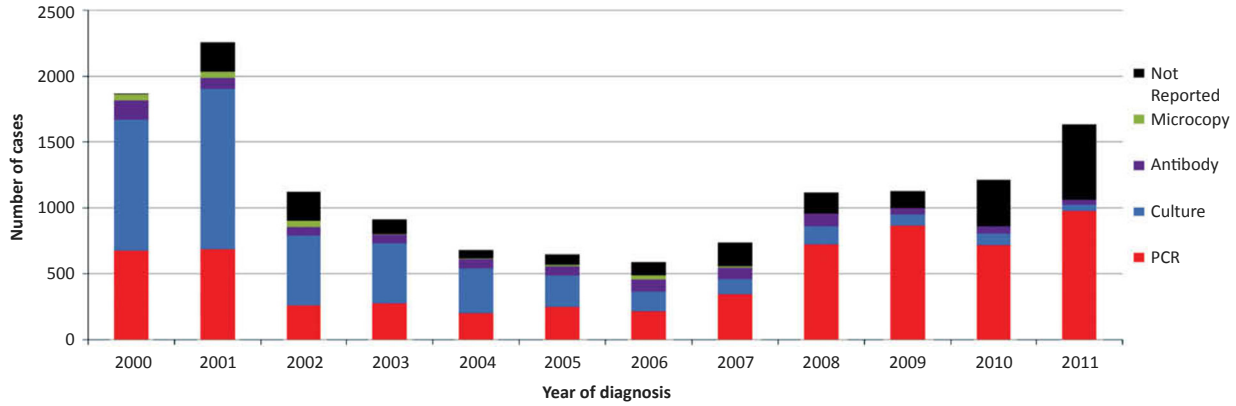

FIG. 3. Changes in the methods used for enterovirus detection in England and Wales during 2000–2011

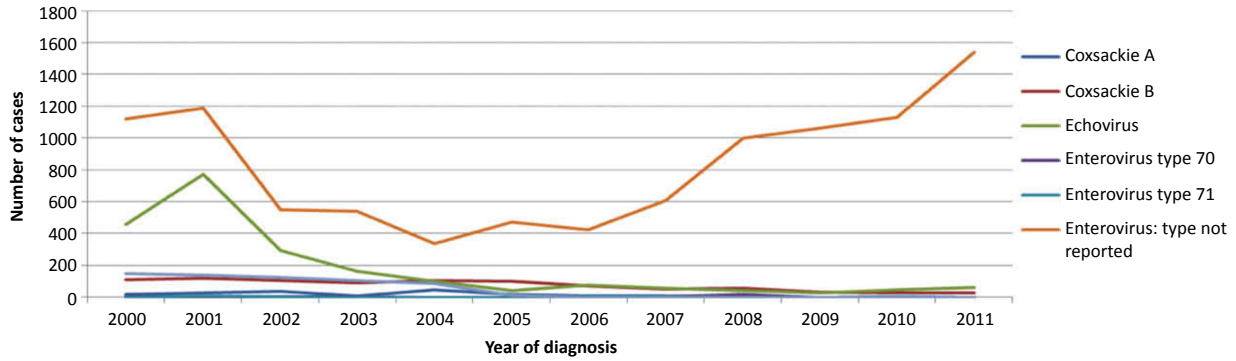

FIG. 4. Enterovirus types (including untyped strains) circulating in England and Wales during 2000–2011, obtained from LabBase2. Note: enterovirus type was not reported because it was not sent to the Virus Reference Department (VRD) or because LabBase2 was not updated after results from the VRD had been reported to the local National Health Service hospital laboratory.

TABLE 2. Enterovirus strains by biological site of detection (no. (%)) submitted to the Public Health England national Virus Reference Department during 2004–2011; biological site was reported for 1875 of 1875 (100%) typed enterovirus strains

	Blood	CSF	Gastrointestinal	Respiratory	Skin	Tissue	Total
Coxsackie A	7 (2)	87 (22)	131 (33)	81 (20)	42 (10)	52 (13)	400 (100)
Coxsackie B	14 (3)	98 (22)	108 (25)	58 (13)	5 (1)	159 (36)	442 (100)
Echovirus	17 (2)	411 (45)	202 (22)	64 (7)	3 (1)	216 (23)	913 (100)
Enterovirus	3 (3)	17 (14)	52 (43)	28 (23)	2 (2)	18 (15)	120 (100)
Total	41 (2)	613 (33)	493 (26)	231 (12)	52 (3)	445 (24)	1875 (100)

laboratories rather than a true increase in disease rates. Among other European countries, during 2000–2007, Finland identified echoviruses 11 and 6 along with Coxsackie A24, B5 and B4 as the five most common EV types circulating [23]. This study included both clinical and environmental samples, and did not describe any temporal trends, but reported that molecular typing of isolates by partial VP1 sequencing was quick and reliable. The study isolated Coxsackie A24 strains, mostly from stool samples obtained from immigrants, and thus thought to be imported infections rather than evidence of endemic circulation. In The Netherlands, national surveillance during 1996–2011 showed a gradual increase in EV-positive stool samples, from 6.5% in 2007 to 10.8% in 2011, with a higher proportion of positive reports being recorded by laboratories using molecular assays than by laboratories using cell culture [24]. The authors noted an increase in untyped reports coinciding with the introduction of molecular EV diagnosis [24]. This is consistent with the findings of our study.

Although the clinical spectra of diseases associated with EV types overlap, some manifestations of EV infection are commonly associated with certain types (e.g. aseptic meningitis

TABLE 3. Enterovirus strains submitted to the Public Health England national Virus Reference Department for confirmation and typing during 2004–2011; untypeable strains were mostly attributable to low sample volumes submitted and/or low viral loads

Year	2004	2005	2006	2007	2008	2009	2010	2011	Total
Total submitted	113	224	359	218	346	412	400	698	2770
Untyped	15	83	162	100	45	64	207	219	895
Typed	98	141	197	118	301	348	193	479	1875
Coxsackie A1	–	–	–	–	1	–	–	–	1
Coxsackie A2	–	–	1	2	5	3	7	11	29
Coxsackie A4	–	2	1	–	8	4	7	5	27
Coxsackie A5	–	–	–	–	3	3	–	–	6
Coxsackie A6	–	1	2	4	8	18	8	34	75
Coxsackie A8	–	–	–	–	–	–	–	4	4
Coxsackie A9	10	7	5	5	24	3	40	3	97
Coxsackie A10	–	–	–	2	4	32	4	4	46
Coxsackie A13	–	–	–	–	1	–	–	–	1
Coxsackie A15	–	–	–	1	–	–	–	–	1
Coxsackie A16	3	3	8	3	8	12	3	47	87
Coxsackie A17	–	–	–	–	–	–	–	1	1
Coxsackie A19	–	–	–	–	1	–	–	1	2
Coxsackie A21	3	1	13	–	–	–	1	–	18
Coxsackie A22	–	1	–	–	–	–	–	–	1
Coxsackie A24	–	1	–	2	–	–	1	–	4
Coxsackie B1	–	–	1	13	1	2	9	5	31
Coxsackie B2	2	7	6	12	18	12	5	45	107
Coxsackie B3	4	26	4	1	39	4	19	4	101
Coxsackie B4	12	4	11	6	13	19	4	40	109
Coxsackie B5	–	33	4	–	4	18	2	33	94
Echovirus 2	–	–	1	–	–	–	–	1	2
Echovirus 3	5	6	–	3	4	–	–	8	26
Echovirus 4	–	–	–	–	1	–	4	–	5
Echovirus 5	4	2	–	–	–	–	–	4	10
Echovirus 6	2	–	39	5	5	18	13	11	93
Echovirus 7	–	10	2	2	11	7	–	42	74
Echovirus 8	–	2	–	–	–	1	–	–	3
Echovirus 9	20	–	–	13	4	38	1	28	104
Echovirus 11	–	–	25	10	8	11	4	58	116
Echovirus 13	–	–	6	–	–	1	1	1	9
Echovirus 14	1	–	–	–	4	–	–	3	8
Echovirus 15	–	–	–	–	–	1	1	–	2
Echovirus 16	12	3	1	–	–	4	–	–	20
Echovirus 17	–	–	1	1	–	3	3	3	11
Echovirus 18	–	1	34	7	10	101	–	16	169
Echovirus 21	–	–	1	–	–	–	–	1	2
Echovirus 25	12	26	4	16	18	6	21	4	107
Echovirus 27	–	1	–	–	1	–	1	–	3
Echovirus 30	–	–	12	9	58	13	3	35	130
Echovirus 31	–	–	–	–	–	1	–	1	2
Echovirus 33	–	–	–	–	–	–	–	17	17
Enterovirus 68	2	–	–	–	–	10	2	–	14
Enterovirus 71	6	3	15	1	37	3	29	8	102
Enterovirus 76	–	–	–	–	–	–	–	1	1
Enterovirus 85	–	1	–	–	–	–	–	–	1
Enterovirus 90	–	–	–	–	2	–	–	–	2

with echovirus 30, hand-foot-and-mouth disease with Coxsackie A16, and acute haemorrhagic conjunctivitis with EV type 70 and Coxsackie A24) [10]. The recent Californian outbreak of EV type 68 over a 100-mile radius may have caused acute flaccid paralysis in up to 20 children over an 18-month period. EV type 68 was isolated from the stools of five children with acute flaccid paralysis, but the lack of active prospective clinical and microbiological surveillance data made it difficult to establish causality [25], emphasizing the importance of active surveillance to monitor emerging serotypes in order to inform public health management. EV type 71 vaccine trials conducted in China, for example, were performed as a consequence of active surveillance identifying an increased burden of EV type 71 disease prompting urgent preventive action, and recent EV type 71 vaccine trials have shown promising safety, efficacy and immunogenicity results in infants and young children [26,27].

The EV incidence in infants aged <3 months was almost 100-fold higher than the overall rate; this is far higher than the rates for bacterial infections in this age group [28,29]. In northern Finland, the annual incidence of bacterial central nervous system infections was 36.3/100 000, as compared with 688/100 000 for viral infection [30]. Comparison of rates is complicated by differences in country-specific clinical practice (e.g. threshold for investigating and hospitalizing patients with suspected viral infections), investigation (e.g. threshold for performing lumbar puncture), and diagnosis (e.g. availability of routine viral cultures or PCR testing). Interestingly, characteristic summer peaks associated with EV infections (cases increasing in April, peaking in July, and declining by September) [8–10] were observed mainly in years with a large number of cases reported, highlighting the importance of considering EV infection throughout the year and not only in the summer months.

Our analysis has some limitations. Comparison of voluntary reporting through LabBase2 with mandatory national healthcare-associated infection reporting systems resulted in an estimated 75% completeness of reporting in 2003, rising to 83% in 2010 [31]. LabBase2 also lacks denominator data, because negative results are not reported. It was therefore not possible to assess the contribution of any changes in clinical practice following national recommendations, such as the NICE guidelines in 2007 encouraging lumbar punctures for young infants with fever (http://guidance.nice.org.uk/CG160). There are also no data on the diagnostic testing offered by NHS laboratories or criteria for EV testing for submitted samples. As a consequence, it was not possible to assess how the availability of different diagnostic tests changed over time, or what proportion of laboratories currently offer routine PCR testing. Another limitation of LabBase2 was the lack of clinical and outcome data. Although isolation of EVs from CSF is likely to indicate viral meningitis, a patient with clinical or laboratory (e.g. CSF lymphocytosis) evidence of viral meningitis but in whom EV was isolated from another site (e.g. throat, stool), for example, may not be identified as a case of EV meningitis in LabBase2.

In conclusion, the increasing trend in EV diagnosis highlights the need to systematically collect more detailed clinical data for laboratory-confirmed cases to provide an evidence base for informing national guidance for the clinical management and investigation of individuals with suspected EV infection. In particular, there is a need to provide higher-quality and more accurate quantitative data on the true burden of EV infections and subsequent long-term outcomes.

Transparency Declaration

The authors declare no conflicts of interest.

External Funding

None.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Enterovirus strains by biological site of detection submitted to the Public Health England national Virus Reference Department during 2004–2011 can be found as supplementary data (Table S1).

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